REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2/7/2017	Final Report	7/30/2015-9/30/2016

4. TITLE AND SUBTITLE

A New Tool for Local Manipulation of Neuronal Micro-Circuitry with Ions and Force.

5a. CONTRACT NUMBER
W911NF-15-2-0056

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Kenneth S. Kosik

E-Mail: kenneth.kosik@lifesci.ucsb.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University of California, Santa Barbara
3227 Cheadle Hall
3rd floor, MC 2050
Santa Barbara, CA 93106-2050

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our goal is to compute the complete functional connectivity map of a living neuronal network spontaneously assembled in culture and determine the utility of its information content as a synthetic memory trace. To date no functional connectivity map exists for living neurons at the resolution proposed here. In fact, a quantitative model of the microcircuitry at the level of the single synapse remains inaccessible with current technology. We will develop a deep learning module for diagnosing MEA patterns and establish a complete connectivity map for hippocampal cultured neurons.

15. SUBJECT TERMS

neurons, memory, connectivity, microcircuitry

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	3	19b. TELEPHONE NUMBER *(include area code)*

UNIVERSITY OF CALIFORNIA Santa Barbara

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

KENNETH S. KOSIK, M.D.
HARRIMAN CHAIR IN NEUROSCIENCE RESEARCH
CO-DIRECTOR: NEUROSCIENCE RESEARCH INSTITUTE
PROFESSOR, DEPT. OF MOLECULAR, CELLULAR & DEVELOPMENTAL BIOLOGY

NEUROSCIENCE RESEARCH INSTITUTE
Santa Barbara, CA 93106-5060
Phone: (805) 893-5222
Fax: (805) 893-2005
http://www.nri.ucsb.edu

February 7th, 2017

RE: Kosik/DARPA Final Report W911NF-15-2-0056

To Whom It May Concern,

Please find attached Final Report for the project titled: "*A New Tool for Local Manipulation of Neuronal Micro-Circuitry with Ions and Force*".

Sincerely,

Kenneth S. Kosik, M.D.

Major Technical Developments and Progress

1. Building the Neural Circuit Probe

The Neural Circuit Probe (NCP) combines probes from Scanning Probe Microscopy with multi-electrode arrays (MEAs). In this first report, we use non-contact probes with feedback based on Scanning Ion Conductance Microscopy (SICM) for mobile electrical recording, local chemical delivery and single cell harvesting from a neural circuit of dissociated hippocampal neurons grown on a commercial MEA composed of 120 electrodes. Mobile electrical recording can assign the signal recorded from the MEA to a specific neuron. Local drug delivery can transiently and reversibly modulate the electrical behavior of individually identified neurons to assess their contributions to the circuit behavior. Individual cell harvesting with a SICM probe can be used to pick up identified cells for single-cell RNA-seq analysis of physiologically defined neurons.

2. Detecting action potential propagation in single axons using multi-electrode arrays

Multi-electrode array (MEA) recording of extracellular action potentials (eAPs) is a high-throughput and non-invasive recording configuration. However, its use precludes knowing the neuronal source of any eAP. In our experiments with cultured mouse hippocampal neurons, we found surprisingly widespread co-occurrence of eAPs among groups of electrodes. Co-active electrode groups had invariant eAP sequences and inter-electrode time delays consistent with action potential propagation in unmyelinated axons. This was confirmed when we manipulated factors known to affect axonal action potential propagation. Co-detection of eAPs by multiple electrodes confirms these eAPs are from individual neurons, allowing us to unambiguously monitor the electrical behavior of single neurons within neuronal ensembles. Our methods revealed details of the expansion and contraction of axonal excitability during circuit development. These propagation signals are also present in cultures of human iPS-derived neurons and thus could be used to study axonal physiology in human disease models.

3. Using the NCP to validate predictions of an inhibitory connection based on a GLM

How external inputs to neurons in a network propagate in it and influence other parts of the network is a crucial step in understanding its internal properties and the way it functions in concert with other parts of the nervous system. We demonstrated that tools from statistical inference can be used to make predictions about how chemical intervention with the activity of neurons can propagate in a network and affect other neurons. We did this by first fitting a Generalized Linear Model (GLM) to spike trains recorded from neurons in a hippocampal culture and inferring effective interactions between these neurons. We then used the fitted

model to perform simulated in silico experiments in which we simulated the effect of silencing individual neurons in a network on the activity of others. We tested the predictions from these simulated silencing experiments by performing real experiments in which we applied Tetrodotoxin (TTX) using the NCP to silence neurons and demonstrate the effectiveness of our approach in detecting inhibitory interactions between pairs of neurons.

To acquire the traces for analysis by the GLM we used Multi-electrode arrays (MEAs) capable of recording extracellular action potentials (eAP) from hundreds of neurons simultaneously. Planer MEAs serve as a substrate for glial and neuronal growth in which neural ensembles self organize over multiple days. The close apposition of neurons with each recording electrode produce recordings with high signal- to-noise properties while the ease of making manipulations in the culture permits access not otherwise possible in vivo. Because cultured neurons self-organize into spontaneously- active synaptic circuits and because neurons fire action potentials primarily in response to synaptic input, recording extracellular action potentials can sample the connectivity phenotypes in large groups of neurons. We discovered that GLM based simulated experiments can identify inhibitory connections.

4. MEA Viewer: a High Performance Interactive Application for Visualizing Electrophysiological Data

Multi-electrode array (MEA) recordings of neuronal signals fill a vital role in the characterization of *in vitro* and *in vivo* neuronal signaling. It is now common to use MEAs to record extracellular action potentials (eAPs) simultaneously from hundreds of neurons. Because of the high sampling frequency required to adequately capture these types of events, the resulting raw data files are large and difficult to visualize with traditional plotting tools. Unbiased data exploration requires the use of tools that minimize data transforms, enabling the development of a heuristic perspective from unprocessed data. We introduce MEA Viewer, a high performance interactive application for the direct visualization of multi-channel electrophysiological data. MEA Viewer provides many high performance visualizations of electrophysiological data, including an easily navigable overview of all recorded extracellular signals overlaid with spike time stamp data and an interactive raster plot. Beyond these fundamental displays MEA Viewer can also extract sub-threshold information, which is information below the spike detection threshold, from single neurons on the array by aligning and averaging eAPs identified by that neuron's propagation signal. This is a direct visualization of both action potential propagation across the plane of the MEA, as well as other events occurring nearby in time such as excitatory post-synaptic potentials (EPSPs) and pre and post-synaptic spiking, thus opening up new and novel research applications for medium density arrays. MEA Viewer is licensed under the General Public License version 3, GPLv3, and is available at http://github.com/dbridges/mea-tools.

5. Construction of patterned arrays for computing neuronal ensemble behavior

We have built a patterned surface that geometrically organizes information flow and reliably controls this flow between two neural ensembles, while simultaneously recoding, visualizing and probing electrical activity within the component neural circuits. This device is illustrated below.